

## **Morphogenesis of Marine Ciliate *Pelagostrobilidium* (Ciliophora: Oligotrichia)**

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### **ABSTRACT**

The morphogenesis of the marine ciliate, *Pelagostrobilidium simile* Song and Bradbury, 1998, was investigated using pyridine silver carbonate impregnation. The morphogenesis of *P. simile* is of hypoapokinetal mode. The oral primordium of *P. simile* commences slightly below the external membranelles (EM) with the proliferation of an anarchic field. Somatic ciliature in proter and opisthe of *P. simile* are derived from the old structure with the proliferation of the basal bodies during the dividing process. Parental oral apparatus of *P. simile* is inherited by the proter, and no reorganization of oral apparatus was observed in the parental oral infraciliature.

Key words: marine ciliate, morphogenesis, oligotrich, *Pelagostrobilidium simile*

### **INTRODUCTION**

Oligotrichs, one of the largest ciliate groups, play an important role in the planktonic food webs in freshwater and marine ecosystems (Lynn and Montagnes, 1988; Müller, 1989; Krainer, 1991; Montagnes and Lynn, 1991; Petz et al., 1995; Song and Packroff, 1997; Montagnes and Humphrey, 1998; Song and Bradbury, 1998; Dolan et al., 1999; Dolan, 2000; Dolan et al., 2002). Recently, the knowledge of the morphogenesis of rare species in this group was updated using protargol impregnation and/or scanning electron microscopy (Deroux, 1974; Petz and

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Foissner, 1992, 1993; Foissner, 1996; Song and Wang, 1997; Dale and Lynn, 1998). The mode of cell division in oligotrichs has been typified as enantiotropic: the axes of the proter and opisthe are more or less distinctive at right-angle to one another and the daughters thus adhere by their posterior portions. The apokinetal morphogenesis is subdivided into epiapokinetal and hypoa-pokinetal. The epiapokinetal oral anlage develops in the cell surface, and the hypoa-pokinetal oral anlage commences in the subsurface pouch or in an intercellular tube. The former was found in halteriids, and the latter was discovered in strombidiids and strobiliids (Foissner, 1996).

Petz and Foissner (1992) defined the spiraling of the somatic kineties at the posterior end as the distinguished characteristics for the genus *Strobilidium*, and one species of the genus *Rimostrombidium*. Partially based on this diagnosis, Petz et al. (1995) erected a new genus *Pelagostrobilidium* to which *Strobilidium neptuni* Montagnes and Taylor, 1994 and *Strobilidium spiralis* (Leegaard, 1915) Lynn and Montagnes, 1988 were transferred, since two species have no such spiral. Song and Bradbury (1998) erected *P. simile* from coastal waters in northern China. The genus *Pelagostrobilidium* was characterized by longitudinal and transversely arched somatic kineties which do not form a spiral at the posterior end (Petz et al., 1995). However, the morphogenesis of this genus has not been described since its establishment. Detailed descriptions of the morphogenetic process of *P. simile* during the asexual binary division are provided in this paper.

## MATERIALS AND METHODS

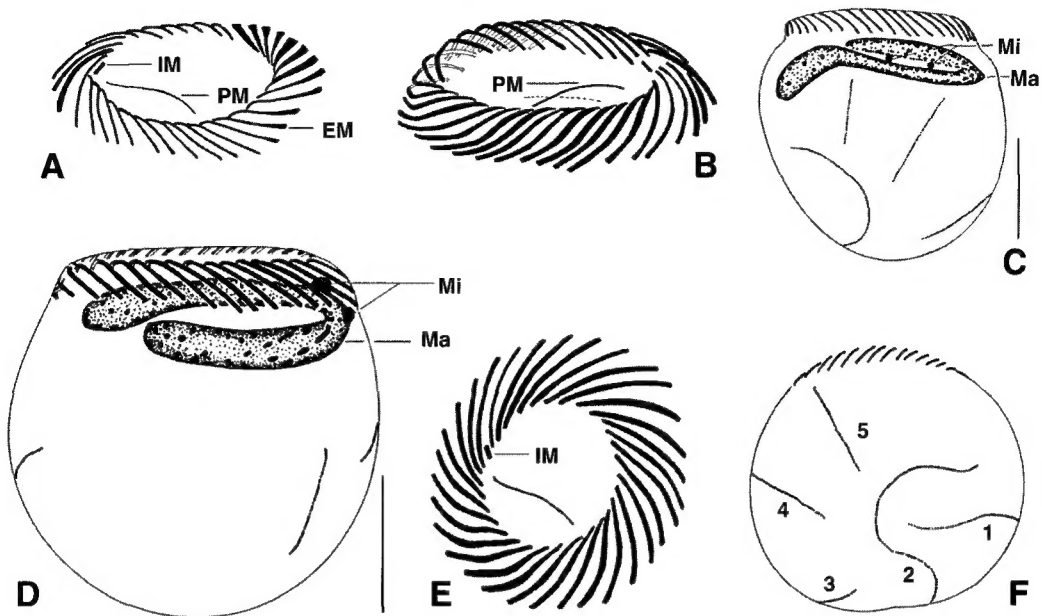
*Pelagostrobilidium simile* was isolated from Incheon coastal waters in Korea (Incheon, 37° 27' N; 126° 35'E) in October 2002. Marine water samples were observed under a dissecting microscope and organisms were collected with a micropipette for live observation and staining.

All specimens were studied *in vivo* and using pyridinated silver carbonate method (Ma et al., 2003) which was moderately modified from the previous method of Fernandez-Galiano (1976). All figures were taken or drawn with the help of a Penguin 600CL digital camera. The morphogenetic events were observed under photomicroscope (Olympus with DIC) and analyzed with anlage structure. The standard terminology is mainly according to Song and Bradbury (1998) and Petz and Foissner (1992).

## RESULTS

### Morphology of nondividing

Cells are subspherical with the anterior end being slightly cylindrical and the posterior end more subconical with a circular transverse section. The cells are 45–65 µm in length and 45–60 µm in width *in vivo*. The cell rotate in one position, disturbed cells detach immediately and dart away 2–4 times of the body lengths. Food vacuoles contain small flagellates or dinoflagellates (2–10 µm). The cytoplasm usually has numerous small glistening globules and vacuoles, which can easily be seen in silver carbonate stains (Fig. 2E). There are 30–34 (32) external membranelles (EM) and one short



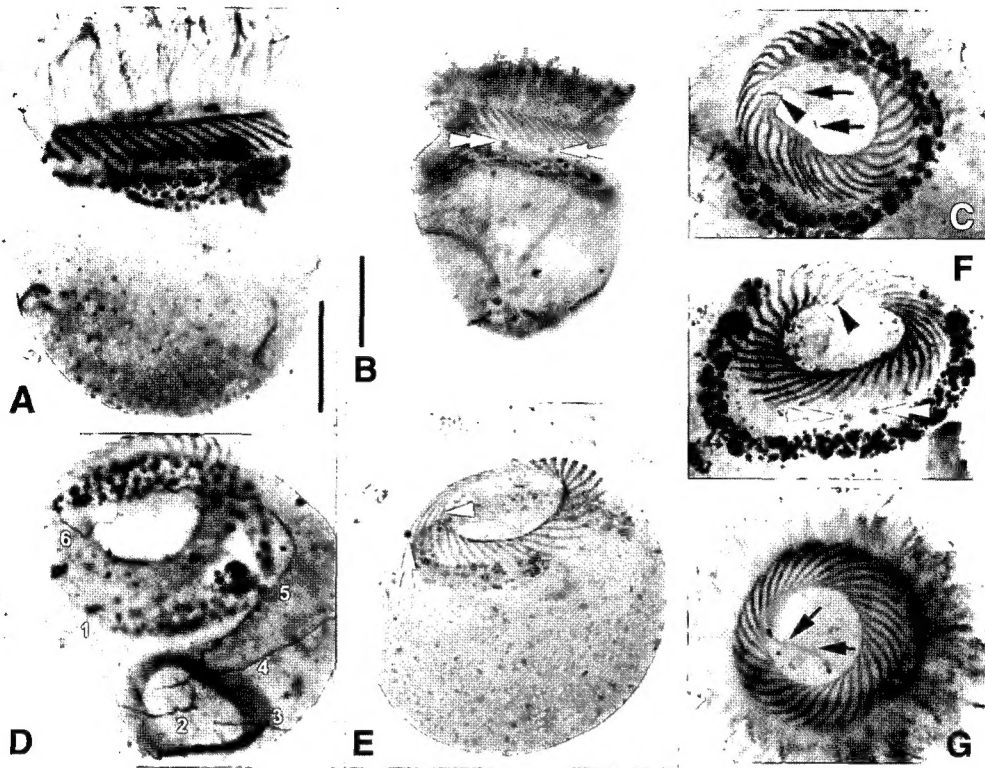
**Fig. 1.** Morphology of *Pelagostrobilidium simile* after silver carbonate impregnation. A-B & E, top view of adoral zone of membranelles; C & D, dorsal or lateral view; F, aboral view. 1-5, somatic kinety 1-5, respectively; EM, external membranelles; IM, internal membranelles; Ma, macronucleus; Mi, micronuclei; PM, paroral membrane. Scale bars: 20  $\mu$ m.

internal membranelles (IM). The paroral membrane (PM) consists of monokinetids, usually not easily seen in impregnated slides (Figs. 1A-B, E; 2C, G). There are five somatic kineties and rarely six (Fig. 2D). The monokinetids are closely arranged and the cilia are very short. Kinety 2 is characteristically arched around the caudal area (Fig. 1F). One horse shoe-shaped macronucleus (Ma) is horizontally located around the oral cavity and below the EM (Figs. 1C-D; 2A-G). Two micronuclei (Mi) are indented into the macronucleus (Figs. 1C-D; 2B, E-F, double arrowheads).

### Morphogenesis of *Pelagosrombidium silmle*

The stomatogenesis commences with the appearance and proliferation of basal bodies just below the external membranelles, which are situated on the dorsal side of the cell between the first and fifth kineties and form an anarchic field (Figs. 3A; 4A, arrow). At the same time, the anarchic field splits into two longitudinally arranged parts (Figs. 3B-C; 4B, arrows and arrowheads respectively), the right one proliferates quickly, and migrates into the subsurface (Figs. 3D; 4C, arrows). Then, the anarchic field forms a pouch structure in the subsurface of the anterior part of the cell, and a separate line appears near the anarchic field (Figs. 3E; 4D, arrow), basal bodies begin to organize membranelle structure.

The next stage is characterized by the curve and migration of the newly developed membranelles in the oral primordium accompanying the spatial development (Figs. 3F-K; 4F-I). The oral primordium assembles itself into 26-34 membranelles and the newly formed membranelles migrate

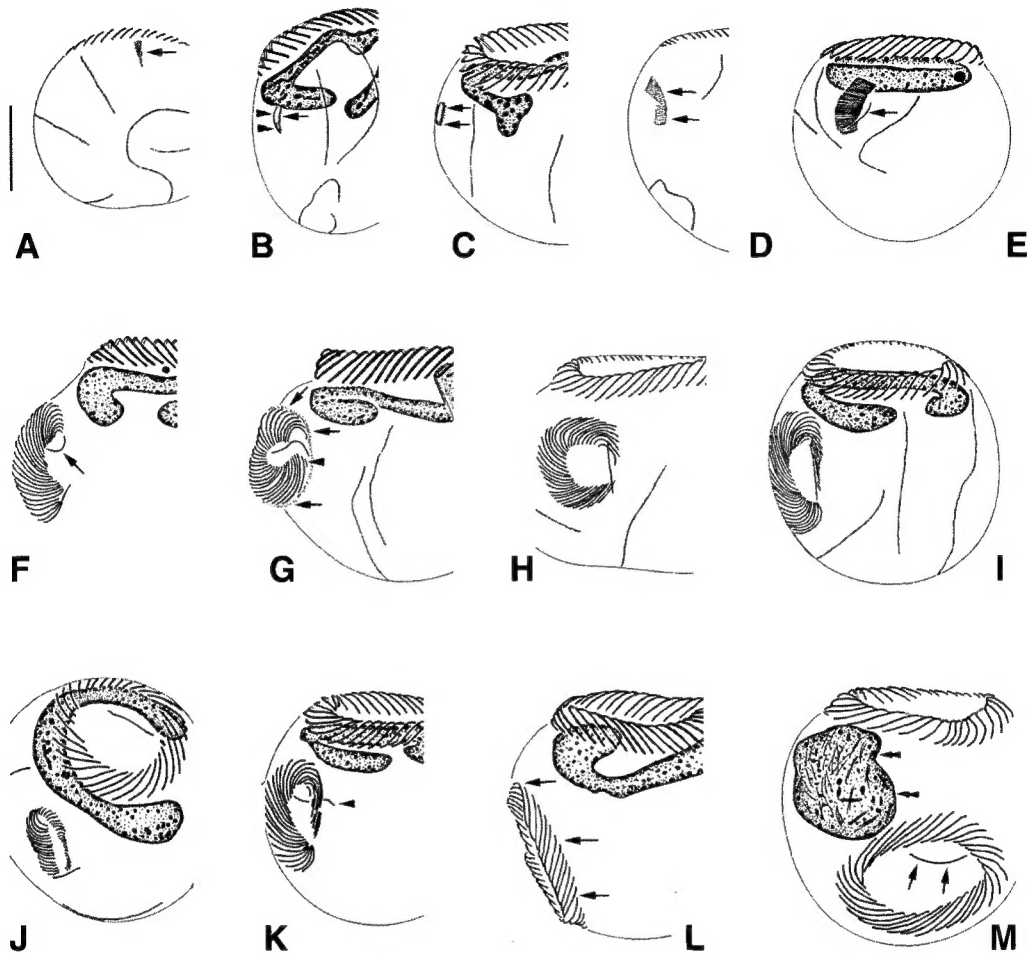


**Fig. 2.** Morphology of *Pelagostrobilidium simile* after silver carbonate impregnation. Arrows indicating the paroral membrane; arrowhead showing the internal membranelle; double arrowheads pointing to the micronuclei; 1-6, somatic kinety 1-6, a very rare situation. Scale bars: 20  $\mu$ m.

downwards and counterclockwise (Figs. 3F; 4H), and the primordium of PM curves slightly (Figs. 3F, 4H, arrow). Then, several sparsely arranged basal bodies commence around the oral primordium (Figs. 3G; 4G, arrows), and meanwhile, a short membranelle which will develop into the IM appears near the internal edge of the external membranelles (Figs. 3G; 4G, arrowhead). The sparsely arranged basal bodies will be absorbed or assemble gradually into the EM. Figures 3H-K, 4F and 4I show the different side view of the same stage. At this stage, the longitudinal axis of the oral primordium is nearly perpendicular to that of the parental oral field.

With the development of the adoral primordium (Figs. 3L; 4E, arrows), the longitudinal axis of the new adoral field migrates downwards and counterclockwise and forms 135° angle with that of the parental adoral field, and the new adoral apparatus bear new cilia (Fig. 4E). At this stage, the reorganization of the macronucleus (Ma) is still not obvious.

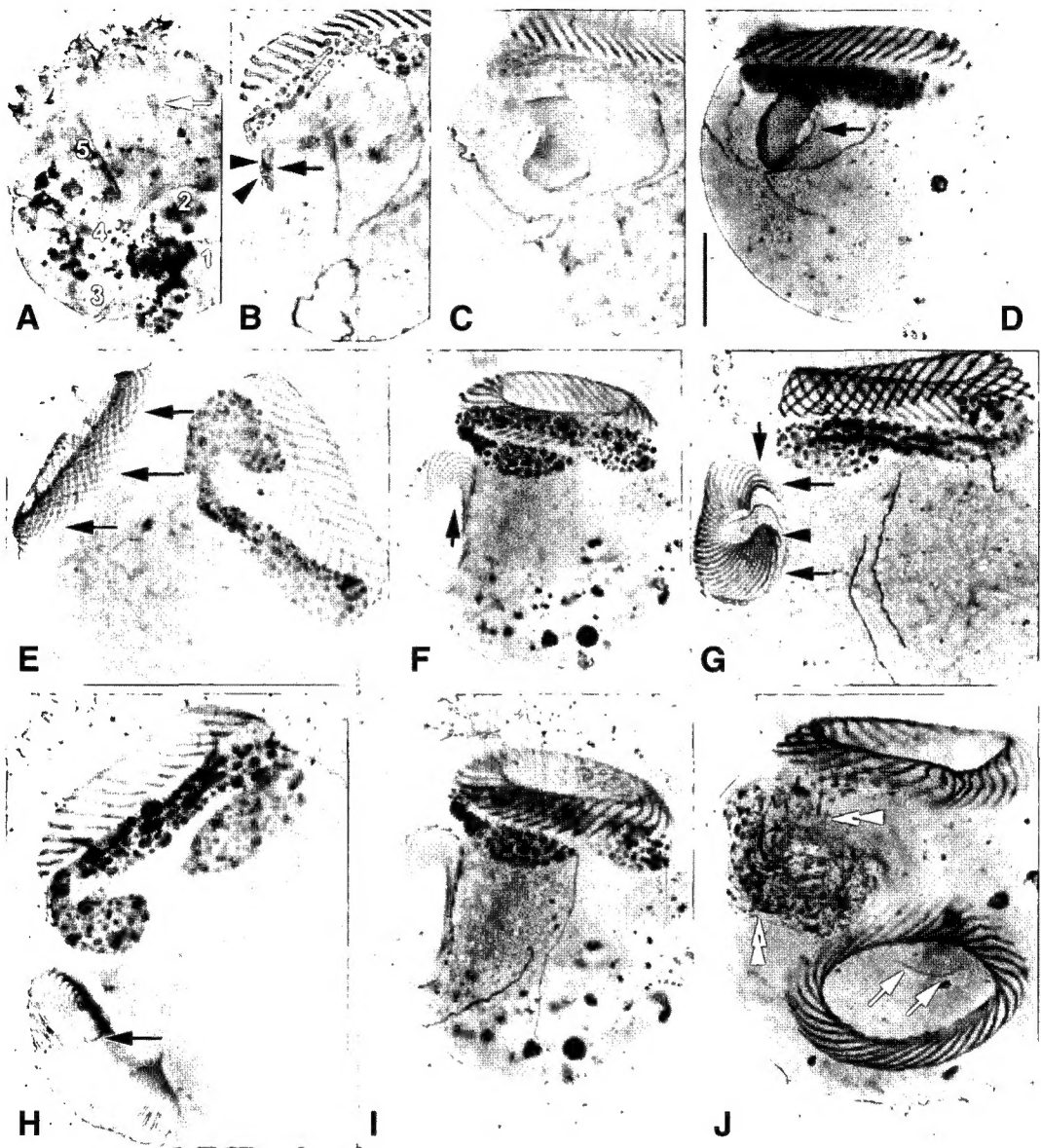
The next stage is characterized by the subsequent reorganization of the Ma, and by the downwards and counterclockwise migration of the new adoral apparatus (Figs. 3M; 4J). The Ma becomes a sphere with large amount of stripe-chord structure (Figs. 3M; 4J, double arrowheads). The longitudinal axis of the new adoral apparatus nearly overlaps that of the parental adoral apparatus and the paroral membrane is clearly observed in the new adoral field (Fig. 3M, 4J,



**Fig. 3.** Morphogenesis of *Pelagostrobilidium simile* after silver carbonate impregnation. A-D, early dividers; E-K, middle stages; L-M, late stages. The arrow in A showing the oral primordium below the external membranelles; arrows and arrowheads in B-C indicating the splitting of the oral primordium; arrows in D pointing to the rapid proliferation of oral primordium; arrow in E-F pointing to the future paroral membrane; arrows in G showing the sparsely arranged basal bodies that will assemble into the external membranelles, arrowhead in G indicating the future internal membranelle; arrow in K pointing to the future paroral membrane; arrows in L showing the adoral zone of membranelles; arrows in M showing the paroral membrane of the opisthe, double arrowheads in M showing the macronucleus that is about to divide. Scale bars: 20  $\mu$ m.

arrows).

Later morphogenetic stages were not found in this study. During the process of morphogenesis, the parental somatic kineties are not resorbed and elongated by intrakinetal proliferation of basal bodies. Only in the late stage they divide into daughter cells. The parental oral apparatus is inherited by the proter, and no reorganization was observed in the parental oral infraciliature.



**Fig. 4.** Morphogenesis of *Pelagostrobilidium simile* after silver carbonate impregnation. A-B, early dividers; C-D, F-I, middle stages; E-J, late stages. The arrow in A showing the oral primordium below the external membranelles; arrows and arrowheads in B indicating the splitting of the oral primordium; arrows in D, F & H each indicating the future paroral membrane; arrows in E showing the adoral zone of membranelles; arrows in G showing the sparsely arranged basal bodies that will assemble into the external membranelles; arrowhead in G indicating the future internal membranelle; arrows in J showing the paroral membrane of the opisthe, double arrowheads in J showing the macronucleus that is about to divide; 1-5, showing the kinety 1-5, respectively. Scale bars: 20  $\mu$ m.

## DISCUSSION

### Morphology of nondividing cells

The Incheon population of *Pelagostrobilidium simile* corresponds well with the population described previously by Song and Bradbury (1998) in the body shape, size, C-Shaped Ma, two micronuclei and the arrangement of somatic kineties. But, it differs from original description only by the number of external membranelles (Incheon population, 30–34; other population, 29–31).

### Morphogenesis

Considering the morphogenetic events, *P. simile* is characterized by the following: the stomatogenesis is of hypoapokinetal (HAK), the adoral primordium commences slightly below the external membranelles on the dorsal side of the cell, the somatic ciliature in both daughters are derived from the old structure with the proliferation of the basal bodies during the dividing process, the parental adoral apparatus (EM, IM, PM) are retained by the proter, and no reorganization was observed in the parental adoral apparatus.

Our observations on *P. simile* suggest that it has some specific characteristics even though the overall division pattern resembles those of *Strobilidium caudatum* and *Strombidinopsis spiniferum* (Petz and Foissner, 1992; Dale and Lynn, 1998). The adoral primordium in *P. simile* commences slightly below the external membranelles and migrates downward to the mid-body during the morphogenetic process, and this is similar in *Strobilidium gyrans* and *Strombidinopsis spiniferum* (Deroux, 1974; Dale and Lynn, 1998), but it does not commence near the mid-body as in *Strobilidium caudatum*. Moreover, the paroral membrane primordium commences earlier than in *Strobilidium* and *Strombidinopsis*. Another characteristic is that the macronucleus is syncretized later in *P. simile* than in *Strobilidium caudatum* and *Strombidinopsis spiniferum* in the process of morphogenesis, but, we could not observed in detail the final stages of cytokinesis because the latter morphogenetic phases are difficult to be found. Ota and Taniguchi (2003) found that most of the parental adoral membranelles of *Pelagostrobilidium* persisted and were active during the entire conjugation period and that the duration of the conjugation process was relatively short.

The morphogenesis of *P. simile* was very similar to that of *Strobilidium caudatum* and *Strombidinopsis spiniferum* described previously (Deroux 1974; Petz and Foissner 1992; Dale and Lynn 1998); but differs from those in Halteriids and in some Strombidiids. The main morphogenetic events in halteriids, e.g., *Meseres corlissi*, *Halteria grandinella*, are apparently identical. The adoral primordium develops on the cell surface and the entire somatic ciliature originates between the parental ciliary rows (Petz and Foissner, 1992; Song, 1993). In some strombidiids (*Pelagostrobilidium fallax*, *Strombidium kryalis* and *Strombidium sulcatum*), the adoral primordium commences from the intracellular tube (Petz and Foissner, 1992; Petz, 1994; Song and Wang, 1997). These characteristics in halteriids and some strombidiids are distinctively different from those of strobilidiids, e.g. *P. simile*, *Strobilidium caudatum*, and *Strombidinopsis spiniferum*.



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해산 섬모충 *Pelagostrobilidium simile* (Ciliophora: Oligotrichia)의 형태 형성

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## 요 약

인천연안에서 채집한 부유성 빈모류인 *Pelagostrobilidium simile*의 형태 형성 과정을 pyridine silver carbonate impregnation 염색법을 이용하여 관찰하였다. *P. simile*의 형태형성은 전형적인 hypoapokinetal mode로 입섬모열은 모세포의 체섬모와 관계 없이 독립적으로 세포막 안에서 외부로 노출되는 입원기로부터 형성되었다. 입원기는 anarchic field의 증식으로 외부 입섬모열 아래에서 시작되었다. 딸세포 proter와 opisthe의 체섬모상은 분열하는 동안 기저체의 증식으로 모세포 체섬모 구조로부터 나왔다. 모세포의 입 기구는 딸세포 proter로 전달되고 모세포의 입섬모구조에서 입기구의 새로운 형성은 관찰되지 않았다.